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A Temperature-Sensitive Mutation Affecting Cilia Regeneration, Nuclear Development, and the Cell Cycle of *Tetrahymena thermophila* Is Rescued by Cytoplasmic Exchange

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A temperature-sensitive mutation was isolated that blocks cilia regeneration and arrests growth in *Tetrahymena thermophila*. Protein and RNA synthesis and ATP production appeared to be largely unaffected at the restrictive temperature, suggesting that the mutation is specific for cilia regeneration and growth. At the restrictive temperature, mutant cells arrested at a specific point in the cell cycle, after macronuclear S phase and shortly before micronuclear mitosis. Arrested cells did not undergo nuclear divisions, DNA replication, or cytokinesis, so the mutation appears to cause true cell cycle arrest. Surprisingly, the mutation does not appear to affect micronuclear mitosis directly but rather some event(s) prior to micronuclear mitosis that must be completed before cells can complete the cell cycle. The cell cycle arrest was transiently complemented by wild-type cytoplasm exchanged during conjugation with a wild-type cell. Each starved, wild-type cell apparently contained enough rescuing factor to support an average of six cell divisions. Thus, this mutation affects assembly and/or function of at least one but not all of the microtubule-based structures in *T. thermophila*.

Tubulin is assembled into a variety of structures that are involved in many cellular functions in eucaryotes. How the assembly of different microtubule systems is regulated and what gives each microtubule system its unique properties remain largely a mystery. To address these questions, we have initiated a genetic analysis of microtubule structures in the ciliated protozoan *Tetrahymena thermophila*.

Microtubules in *Tetrahymena* are known to be involved in cell movement and feeding (cilia and the associated basal bodies and ciliary rows), nuclear divisions (both macronuclear amitotic division and micronuclear mitosis), maintenance of cell shape (cortex), and nuclear movements and divisions during conjugation (1, 11, 19, 24, 25, 27, 35). The diverse microtubule structures found in *Tetrahymena* are constructed by using five α-tubulin isoforms (three localized to the cilia, two to the cytoplasm) and two β-tubulin isoforms (both are found in the cilia, one in the cytoplasm) (32). *Tetrahymena* have only one α- and two β-tubulin genes (6, 9; G. Shalke and M. A. Gorovsky, unpublished observations), and the numerous tubulin isoforms in *Tetrahymena* are likely to be produced by the same posttranslational modifications that occur in higher eucaryotes (28). *Tetrahymena* is amenable to conventional genetic analysis (see reference 7 for a review) and can regenerate a full complement of cilia within 3 h after mechanical deciliation (10, 29).

This report describes the characterization of a class of mutant *Tetrahymena thermophila* that fail to regenerate cilia at 38°C. At this temperature, these mutants also arrest both nuclear and cytoplasmic events at a specific point in the cell cycle. When mutant cells are mated to each other and incubated at 38°C, nuclear divisions occur, but nuclear development is abnormal. The two phenotypes, division arrest and failure of cilia regeneration, are tightly linked. Both growth arrest and abnormal nuclear development during conjugation can be rescued by transfer of wild-type cytoplasm during conjugation. Thus, we have isolated a unique mutation that appears to affect biosynthesis, assembly, or function of some but not all microtubule systems in *Tetrahymena* and which also interacts with the nuclear replication cycle.

MATERIALS AND METHODS

Strains, nomenclature, media, and culture conditions. Strains Cu428 and A*III were kindly provided by Peter Bruns (Cornell University). Cu428 is a functional heterokaryon that is homozygous for the allele carrying resistance to 6-methylpurine (6mp) in the micronucleus and expressing only the allele that confers sensitivity to 6mp in the macronucleus (8). Strain A*III is a strain that undergoes genomic exclusion; it has a defective micronucleus that does not participate in nuclear development during conjugation (4, 5).

Nomenclature is that proposed by Bruns and Brussard (8) in which the micronuclear genotype is listed first, followed by the phenotype in parentheses. Cu428 is designated *6Mpr/6Mpr* ($6mp^s$ VII). *6Mpr* is a dominant mutation conferring resistance to 6mp (15 μg/ml) so, as described above, the strain is genotypically resistant but phenotypically sensitive to 6mp and is mating type VII.

All strains were cultured in a modified Neff medium that contained all the salts but 1/3 the amount of proteose peptone (Oxoid), yeast extract (Difco Laboratories), and glucose (Baker) found in regular Neff medium (14). Fungazone (Gibco) at 2.5 μg/ml and penicillin-streptomycin (Gibco) at 100 U and 100 μg/ml, respectively, were routinely added to the growth medium. Cells were grown in sterile flasks with shaking (approximately 100 rpm) at 28 to 30°C. Cells were starved and mated in 10 mM Tris hydrochloride pH 7.4, as described (20).

Cell manipulation and drop culture. Single cells and mating pairs were isolated and manipulated by techniques and equipment described in Orias and Bruns (26). Drop culture and microtiter plate culture were as described (26).

Deciliation. Cells were deciliated and incubated for cilia

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regeneration as described (10) except that cells were swirled very gently during deciliation and were incubated without shaking during cilia regeneration.

Microscopy. Cells were fixed for microscopy by the addition of either formaldehyde (Baker, 37%) to a final concentration of 1% or modified Schaudin (2 parts saturated $HgCl_2$, 1 part 99% ethanol, 0.01 volume glacial acetic acid). Cells were observed with an Olympus BH-2 compound microscope equipped with phase-contrast and Nomarski optics and fluorescence. For cell morphology, cells were observed with Nomarski optics. For nuclear morphology, nuclei were stained with 0.001 mg of 4'-6-diamidino-2-phenyldihydrochloride (DAPI; Polysciences) per ml and viewed with UV light.

Calculations. To calculate the volumes of mutant cells at 28 and 38°C, we used the formula for calculating the volume V of a prolate spheriod, $V = 4/3\ \pi ab^2$, where a is half the length and b is half the width. We determined the lengths and widths of 51 SG71 cells and 54 SG68 cells incubated at 28°C and 50 SG71 cells and 46 SG68 cells incubated at 38°C. The calculated volumes were averaged to obtain the values given in the text.

The execution point (EP) of the group 5 mutation was determined by isolating single cells from a log-phase culture of SG68 cells into drops of conditioned medium and incubating the cells at 38°C for 2.5 h. The number of drops in which cells had divided was determined. To correct the execution point for the fact that a logarithmically growing culture contains twice as many cells that just completed cytokinesis as cells just entering cytokinesis, we used the formula given in Frankel (15): $F = [\ln (R + 1)]/\ln 2$, where R is the fraction of cells seen in some terminal phase of the cellular cycle, and F is the fraction of the generation time spent in this phase. Thus, the measured EP = 100% − 100R, and the corrected EP = 100% − 100F.

[^{3}H] thymidine labeling and autoradiography. Cultures of mutant or wild-type cells in logarithmic growth were divided into two parts, and [^{3}H]thymidine (Amersham, 93 Ci/mmol) was added to each to a final concentration of 20 μCi/ml. One of each pair of cultures was immediately shifted to 38°C, and one was left at 28°C. All cultures were incubated with shaking for 2.5 h, the equivalent of about one generation, and were then fixed with modified Schaudin. Cells were dropped onto poly-L-lysine-coated microscope slides and prepared for autoradiography by dipping into 10% trichloroacetic acid–0.1% iodine in 70% ethanol, dehydrating in ethanol, and dipping dehydrated slides into photographic emulsion. Slides were exposed at 4°C.

RESULTS

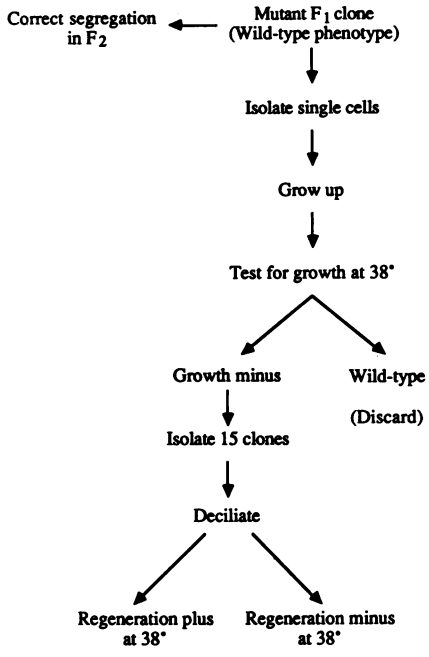
Isolation and genetics of mutants. In a single screen, we isolated 30 temperature-sensitive mutants of *T. thermophila* that neither regenerated cilia normally nor grew at the restrictive temperature of 38°C (Pennock et al., submitted). The mutants were designated SG for slow growth. The SG phenotype in most of the fertile mutants behaved as if caused by a single, recessive gene, and the mutations were mapped to five complementation groups. The group discussed in this report (group 5) contained fourteen members (SG24, -66, -67, -68, -69, -70, -71, -72, -73, -74, -75, -76, -77, and -79). The levels of total protein synthesis and the levels of RNA encoding α-tubulin and actin during growth at 38°C were similar in mutant cells and wild-type cells, and nondeciliated cells remained motile at 38°C (Pennock et al., submitted). Thus, the mutation(s) in complementation group 5 appears to affect cilia regeneration and growth specifically. A more detailed analysis of this group of mutants seemed warranted.

Cilia regeneration and slow growth are linked in the macronucleus. Previous work showed that the two phenotypes exhibited by the group 5 mutants are meiotically linked in the micronucleus (Pennock et al., submitted), but too few F_2 progeny were analyzed to demonstrate tight linkage. Before we engaged in further characterization of these mutants, we wanted to have greater confidence that the slow growth and cilia regeneration phenotypes were caused by a single mutation. *Tetrahymena* macronuclei divide amitotically, so heterozygotes eventually become homozygous in the macronucleus for one of the two alleles but remain heterozygous in the micronucleus. This process has been termed phenotypic assortment (31). Several loci that are genetically linked in the micronucleus (2, 12, 21) are unlinked in macronuclei, i.e., they do not assort together (3, 7, 12, 13). Thus, if the slow growth and cilia regeneration phenotypes assort together, they have to be tightly linked, and the probability that both phenotypes are the result of the same mutation would be greatly increased.

Single cells from an F_1 synclone (isolated from an SG68 × Cu428 mating) were isolated, expanded, and assayed for ability to grow at 38°C. Fifteen clones that did not grow well at 38°C (had assorted to the SG phenotype) were expanded and starved. The 15 cultures were divided into three groups of five, and equal numbers of cells from each of the five were mixed, deciliated, and regenerated at 28 or 38°C. Fewer than 5% of the cells were motile in either of the two pools of clones after regeneration at 38°C (Fig. 1). Thus, in all 15 cases, both phenotypes assorted together. Chi-square analysis yielded a P value of <0.001, indicating that both phenotypes are linked in the macronucleus.

Cilia regeneration in group 5 cells is delayed at 38°C. Three members of complementation group 5 (SG79, SG75, and SG68) and wild-type cells were starved, deciliated, and incubated at 28 or 38°C. Samples of cells were taken every 30 min, and the percent motile cells was calculated. Wild-type cells showed similar rates and extents of cilia regeneration at 28 and 38°C (data not shown). At 90 min postdeciliation, all of the cells in all three mutant strains incubated at 28°C were motile, while no motile cells were observed in any of the mutant cultures incubated at 38°C. At 3 h postdeciliation, more than 95% of the cells at 28°C had regenerated a full complement of cilia. In three cultures incubated at the nonpermissive temperature, approximately 50% of the cells had sparse, short cilia but made only twitching movements. After 6 h of incubation at 38°C, 50 to 100% of the mutant cells had regained motility. Thus, cilia regeneration in group 5 mutants was delayed, not completely inhibited.

Morphology of growth-arrested group 5 cells. Cultures of two different group 5 mutants (SG71 and SG68) in early log phase of growth (10^5 cells per ml) at 28°C were shifted to 38°C, incubated for 4 h, fixed, and observed with phase and Nomarski optics. At 38°C mutant cells appeared rounder and fatter than at 28°C (Fig. 2). The volume of cells incubated at 38°C averaged 3.1 times that of cells incubated at 28°C. Mutants incubated at 38°C were 1.3 times longer and 1.6 times wider and had a length/width (L/W) ratio about 0.8 that of cells at 28°C. Unlike cultures of mutant cells at 28°C, cultures at 38°C did not contain dividing cells. These observations suggest that mutants incubated at the restrictive temperature arrested at some point in the cell cycle prior to cytokinesis. This possibility was supported by the growth pattern of cultures of mutant cells incubated at 38°C. When group 5 cells in the early log phase of growth were shifted to



	N	N	χ^2	P
Group 5 (SG 79)	0	15	15.00	<0.001

FIG. 1. Macronuclear linkage of slow growth and cilia regeneration phenotypes. Clones derived from a single F_1 synclone heterozygous for the slow growth mutation were tested for growth at 38°C. Fifteen F_1 clones that had assorted to the slow growth phenotype were starved and divided into three groups of five. To determine whether the clones in each group had also assorted to cilia regeneration-negative, equal numbers of starved cells from each member of the group were deciliated en masse and allowed to regenerate for 3 h at 28 or 38°C. All the clones in a group were considered to be cilia regeneration-negative if fewer than 5% of the cells incubated at 38°C were motile and more than 95% of the cells incubated at 28°C were motile.

38°C, they showed an early slight increase in cell number, after which the cell number remained relatively constant for at least 6 h (Pennock et al., submitted). We did not undertake a detailed study of normal growth-related ciliogenesis in mutants incubated at 38°C, but arrested cells did not contain a new oral apparatus in the posterior of the cell when examined with Nomarski optics (data not shown).

Mutant cells (SG79) incubated at 28 or 38°C for 4 h were fixed, stained with DAPI, and examined with the fluorescence microscope. All mutant cells at 38°C had a single, large, irregular macronucleus that stained less intensely than the smaller macronucleus seen in the mutant cells incubated at 28°C (Fig. 2). More interestingly, fewer than 3% of the cells incubated at 38°C had dividing micronuclei (n = 135), whereas 22% of cells incubated at 28°C were in the process of division and contained divided or dividing micronuclei (n = 200). These observations suggest that the terminal arrest point of mutant cells incubated at 38°C is prior to micronuclear division and that, after the temperature shift, mutant cells in the process of micronuclear mitosis completed mitosis, macronuclear division, and cytokinesis and then arrested in the next cell cycle.

EP of the group 5 mutation is at 78% of the cell cycle. Mutants incubated at 38°C arrested with similar terminal phenotypes. Observations of nuclei in arrested mutants suggest that cells late in cell cycle, when shifted to the restrictive temperature, completed the cell cycle and divided before arresting. Analysis of growth at 38°C (Pennock et al., submitted) indicated that only a small percentage of mutants in a log-phase culture divided. These data suggest that the group 5 gene product is required during only part of the cell cycle and that progression through the cell cycle is blocked at 38°C. To determine whether the group 5 gene product is required throughout the cell cycle, we isolated single cells from a culture of mutants (SG68) in log growth at 28°C, placed them into drops of conditioned medium, and incubated the single cells at 38°C for the equivalent of one cell cycle (2.5 h). Essentially all of the wild-type cells treated as described above divided at least once at 38°C, while only 22% of the 312 mutant cells at 38°C divided. These results suggest that only 22% of the cells in a log-phase culture are past the last point in the cell cycle where the group 5 gene product (or some other substance whose synthesis or activation requires the group 5 gene product) is required. Thus, the uncorrected EP (17) of the mutation is at the 78% point of the cell cycle. These results are consistent with previous results indicating that cells late in the cell cycle at the time of the temperature shift divided while cells early in the cell cycle did not. In a logarithmically growing culture, each old cell divides into two young cells, resulting in a culture containing twice as many young as old cells (see reference 22 for a discussion of cell cycle analyses). After correcting for this fact (see Materials and Methods for the correction formula), the EP for the group 5 mutation is at the 71% point of the cell cycle. However, since the EP will be compared with other cell cycle measurements that have not been similarly corrected (see below), we shall continue to refer to the uncorrected value of 78%.

Mutant cells at 38°C arrest after macronuclear S phase and before micronuclear S phase. Mutant (SG79) and wild-type cells in log growth were shifted to 28 or 38°C, labeled with [^{3}H]thymidine for the equivalent of one generation (2.5 h), and examined by autoradiography. All of the nuclei in wild-type cells were heavily labeled at both 28 and 38°C (data not shown). All nuclei of mutant cells incubated at 28°C were also heavily labeled (data not shown). However, only about 77% of the macronuclei were labeled in mutant cells incubated at 38°C (n = 380), and the intensity of labeling was variable (Fig. 3). These results suggest that cells in macronuclear G1 or macronuclear S phase, when shifted to 38°C, must have completed macronuclear S, arrested in macronuclear G2 prior to micronuclear mitosis, and then failed to initiate a new round of DNA replication. The 23% of the mutant cells incubated at 38°C in which the macronuclei were not labeled must have completed macronuclear DNA replication and been between macronuclear S and the EP at the time of the temperature shift. Other experiments (36) place the end of macronuclear S phase at about the 54% of the cell cycle. Thus, this experiment independently places the EP of the mutation at the 77% point of the cell cycle (54% = end of macronuclear S + 23% cells with unlabeled macronuclei), a point very close to the 78% point calculated by counting the number of cells in a log-phase culture that divided after being shifted to 38°C.

Micronuclear S phase actually begins during cytokinesis (which begins after the EP of the group 5 mutation) and continues into the beginning of the next cell cycle (36). In the 190 cells in which micronuclear labeling was assessed, 22% of the cells had labeled micronuclei, and 88% of those also had labeled macronuclei, which is consistent with our hy-

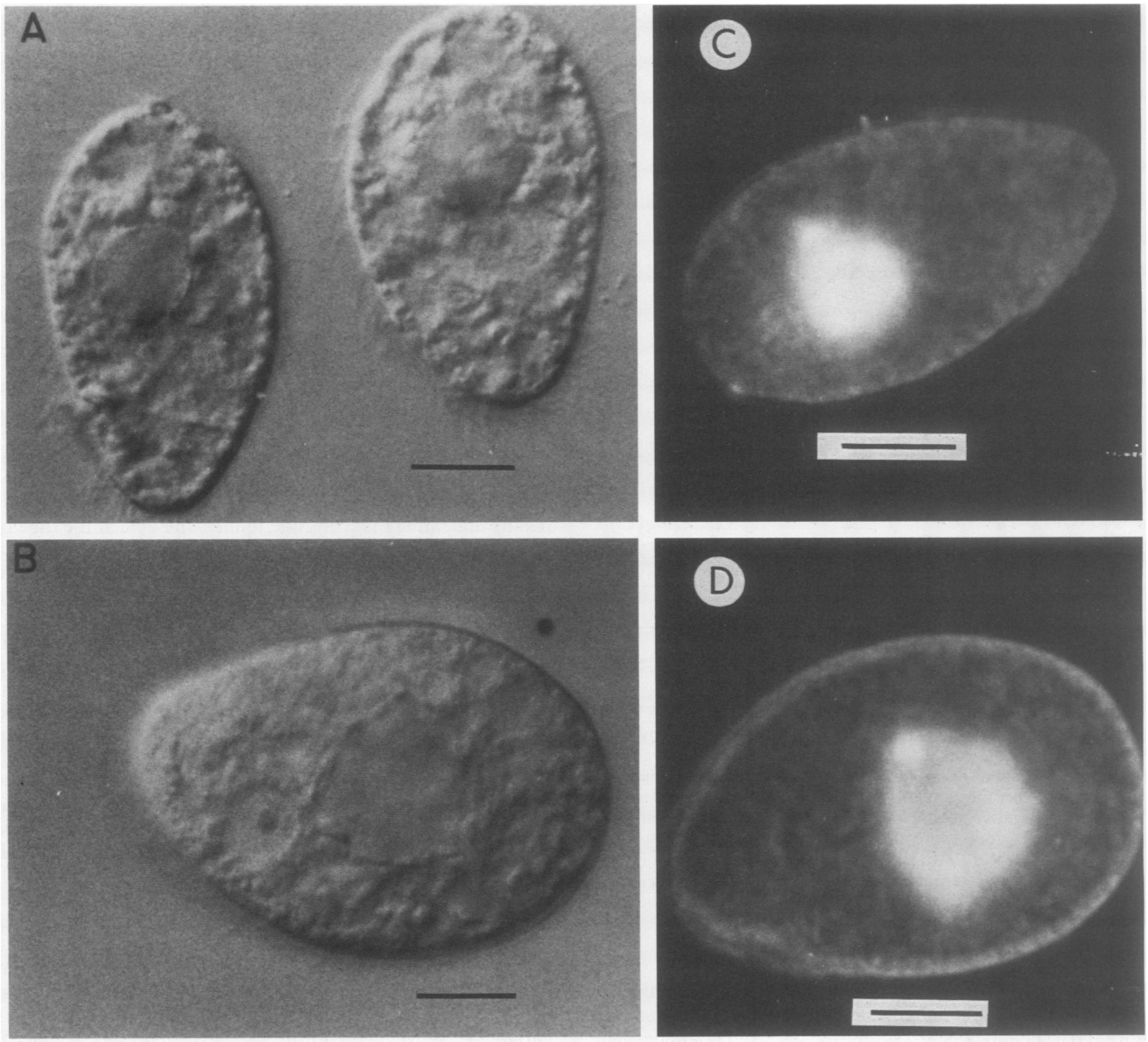


FIG. 2. Cellular and nuclear morphology of group 5 mutants. A culture of SG79 cells in logarithmic growth was incubated at 28 or 38°C for 4 h and fixed with formaldehyde. (A, B) Cells were incubated at 28°C (A) or 38°C (B) and observed with Nomarski optics. (C, D) Cells were incubated at 28°C (C) or 38°C (D), stained with DAPI, and illuminated with UV light. Bars, 10 μm.

pothesis that cells in micronuclear S phase at the time of the shift to 38°C completed micronuclear DNA synthesis, entered and completed macronuclear S phase, and then arrested. We do not know why a small number of cells (approximately 2% of the total) had a labeled micronucleus and an unlabeled macronucleus, but it seems likely that micronuclear S is triggered in a small percentage of arrested cells in the absence of micronuclear mitosis. Note that the determinations of the two S phases (36) are not corrected for the fact that an exponentially growing culture has twice as many young as old cells. These results indicate that the group 5 mutation causes true cell cycle arrest (DNA replication, nuclear divisions, and cytokinesis do not continue in arrested cells) and that cells arrest in macronuclear G2 prior to the 78% point of the cell cycle.

Group 5 mutation causes abnormal nuclear development during conjugation. Group 5 mutants have defects in cilia regeneration and arrest during growth near the 78% point of the cell cycle. Oral development and micronuclear mitosis normally begin at about that point (16, 23, 36). Both mouth formation and mitosis require microtubule assembly and fail to occur in arrested cells. The group 5 mutation could affect either or both processes. To test whether the mutation directly affects micronuclear mitosis, we examined nuclear development during conjugation in mutant cells. During conjugation in *Tetrahymena*, micronuclei undergo a precise series of meiotic and mitotic divisions and nuclear movements leading to fertilization and nuclear differentiation to yield two new micronuclei, two new macronuclei, and a degenerating old macronucleus, all of which are visible in exconjugant cells (see Orias [25] for a review). A mutation that blocks mitosis during vegetative growth should also affect nuclear division during conjugation at the nonpermissive temperature.

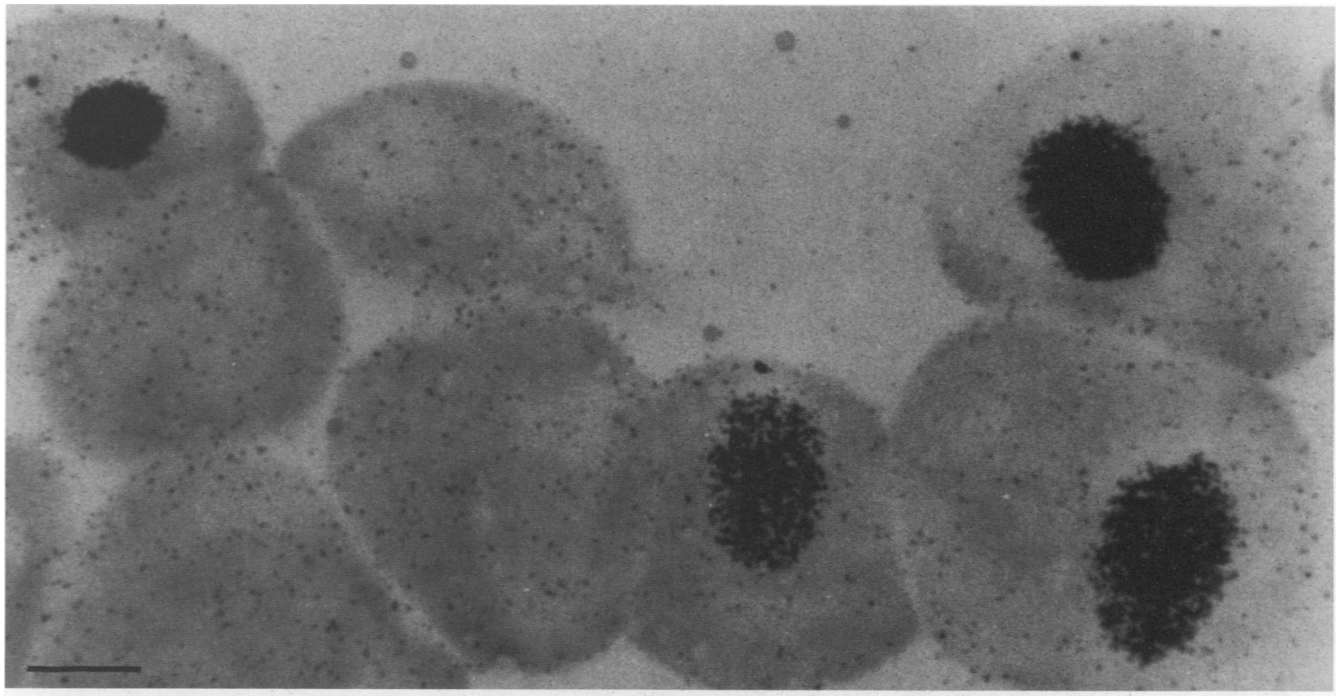


FIG. 3. [^{3}H]thymidine incorporation into mutant cells at 38°C. [^{3}H]thymidine (final concentration, 10 μCi/ml) was added to a culture of SG79 cells in logarithmic growth. Half of the culture was immediately shifted to 38°C and incubated for 2.5 h (equivalent of one generation); the remaining half was incubated for 2.5 h at 28°C. After incubation, cells were fixed with modified Schaudin, spotted onto microscope slides, and prepared for autoradiography. After autoradiography, cells were stained with 1% Giemsa (Fisher), washed, and air dried. The cells shown in the figure were incubated at 38°C during labeling. The slide was exposed for 4 days at 4°C. Bar, 20 μm.

Two group 5 mutants with different mating types were starved and mated as usual (20) except that cells were incubated at 22 rather than 30°C to allow clear distinction between permissive and nonpermissive conditions. Approximately 4 h after mixing, when most paired cells were in the premeiotic crescent stage, growth medium was added to prevent further pairing, and part of the culture was shifted to 38°C. Wild-type cells of different mating types and mixtures of wild-type and mutant cells were treated the same way. Mutant cells did not pair with each other or with wild-type cells very well (perhaps the mutation interferes with pairing), and in several attempts the best pairing we observed had fewer than 50% of the cells in pairs. After incubation, overnight samples from all cultures were fixed, stained with DAPI, and examined with UV light. It should be added at this point that incubation at 38°C causes aberrant macronuclear development and is ultimately lethal to conjugating *T. thermophila* (30), but the nuclear divisions and movements that occur before the critical period of macronuclear development take place normally at 38°C (30) (Fig. 4).

At 22°C nuclear development in mutant cells resembled that in normal cells (Fig. 4A). Cells seemed to separate normally, and the old macronucleus, new micronuclei, and new macronuclei could be identified in exconjugants. However, almost every exconjugant appeared to have one or more extra DAPI-staining bodies (arrow in Fig. 4A) not usually observed in wild-type cells. Whatever its origin, this extra DNA-containing body did not appear to have an adverse effect on conjugation, as genetic segregation in mutants was normal (Pennock et al., submitted).

Mutant pairs incubated at 38°C showed an abnormal phenotype (Fig. 4B). Cells remained paired, and both members of the pair contained multiple nuclei. The nuclei were variable in shape and staining intensity, and we could not distinguish macronuclear anlagen or new micronuclei with certainty. However, the old macronucleus was readily identified. The number of nuclei in each cell was variable. These results suggest that nuclear division can occur in mutants conjugating at 38°C but that nuclear development is grossly abnormal. During normal conjugation, nuclei move to specific regions of the cell, and the fate of each nucleus appears to be determined by its position in the cytoplasm (see Orias [25] for a review). Shortly after the second meiotic nuclear division, three nuclei move to the rear of the cell, do not divide again, and are degraded. If in the mutant pairs at 38°C the nuclei fail to migrate to the posterior and degrade, but instead remain at the anterior and continue to divide, a multinucleate phenotype would be expected. To determine whether nuclei in mutant conjugants at 38°C divided normally but failed to migrate to their correct positions in the cell, we examined mutant pairs isolated at relatively early times after the shift to 38°C. Pairs were observed with multiple dividing nuclei and few or no nuclei in the posterior of the cell (Fig. 4C). See Martindale et al. (20) for a description of nuclear behavior during normal conjugation. After 2 to 3 h at 38°C, most mutant pairs had multiple nuclei, and nuclei could often be observed in the posterior of the cell. We also observed pairs that appeared to be exchanging nuclei when fixed. Since these pairs also had multiple nuclei, this observation suggests that the apparatus involved in exchanging nuclei during conjugation is unaffected by the group 5 mutation, while the machinery that moves nuclei around within a cell requires functioning group 5 gene product.

These results indicate that nuclei divide in conjugating pairs of mutant cells incubated at 38°C but do not migrate

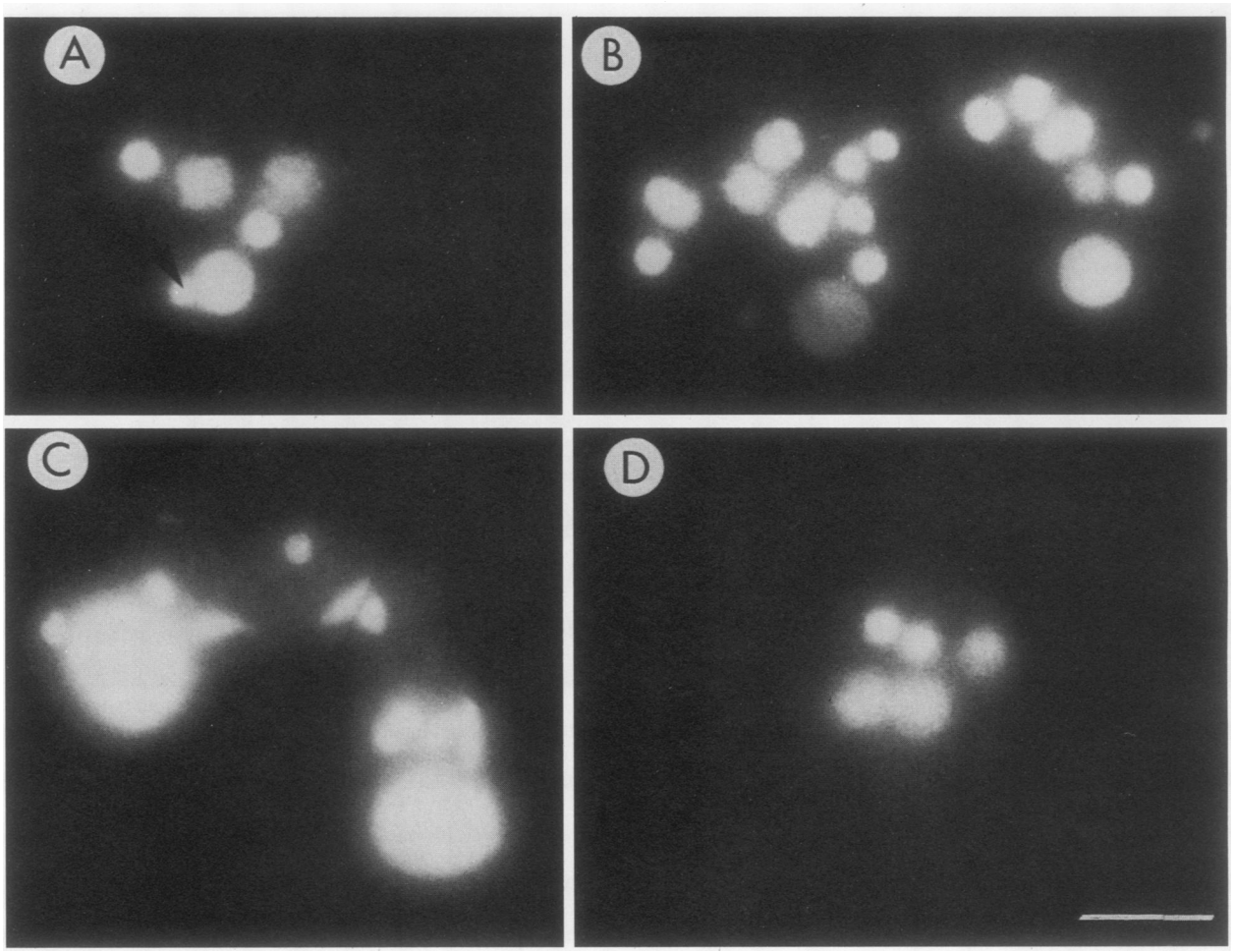


FIG. 4. Nuclear development during conjugation at 38°C. SG79, SG75, and wild-type (Cu428) cells were starved, mated in various combinations, and incubated at 22°C until the majority of pairs were in the premeiotic crescent stage. Cultures were fed to prevent further pairing and shifted to 38°C or left at 22°C. Samples were taken at various times and fixed in formaldehyde. Fixed cells were stained with DAPI and illuminated with UV light. The exconjugant shown in panel A is from an SG79 × SG75 mating after incubation overnight at 22°C. The arrow is pointing to an extra DAPI-staining body. The pair shown in panel B is from an SG79 × SG75 mating after incubation overnight at 38°C. The pair shown in panel C is from an SG79 × SG75 mating after incubation for 2.5 h at 38°C. The cell shown in panel D is an exconjugant from an SG79 × Cu428 mating after incubation overnight at 38°C. Bar, 10 μm.

normally and thus escape their normal fates. Therefore, it seems likely that the mutation does not affect the mitotic apparatus in vegetative cells directly but rather affects some event that is necessary for the occurrence of mitosis. During normal micronuclear division, the micronucleus leaves its position adjacent to the macronucleus, migrates to the periphery, and divides. The group 5 phenotype could be explained if micronuclear movement is a prerequisite for micronuclear mitosis and cannot occur in mutant cells incubated at 38°C.

Wild-type cytoplasm rescues the defect in nuclear development in mutant cells. Surprisingly, conjugation proceeded normally in mutant cells at 38°C when mutant cells were paired with wild-type cells (Fig. 4D). We observed no pairs or exconjugants with phenotypes similar to those exhibited by conjugating mutants at 38°C. In fact, mutant × wild-type pairs incubated at 38°C did not contain the extra DAPI-staining bodies we observed in mutant × mutant pairs incubated at 22°C. Thus, the wild-type group 5 gene product, or some molecule whose synthesis or activation requires that gene product, must have diffused from the wild-type conjugant into the mutant member of the pair and allowed processes normally affected by the group 5 mutation to proceed normally.

Wild-type cytoplasm rescues cell cycle arrest in mutant cells. We wanted to ascertain whether the cell cycle arrest at 38°C exhibited by mutant cells could also be rescued by wild-type cytoplasm. For this purpose, mutant × wild-type matings would not work because conjugation results in reciprocal fertilization, and the new macronuclei (and micronuclei) of both cells would be heterozygous and the exconjugant would be phenotypcially wild type. To overcome this problem, mutant cells were mated with "star" (*) strain cells that have wild-type macronuclei and cytoplasm but a defective micronucleus. When cells containing a normal micronucleus are mated with cells of a star strain, the micronucleus in the normal cell produces a normal migratory and stationary gametic nucleus, and donates a gametic nucleus to the star cell. On the other hand, the star micronucleus is defective, and the star cell fails to donate a nucleus to its mate. The cells then separate, and except for diploidization of the new micronucleus derived from the nonstar cell, no

further nuclear development occurs. Thus, in this process, referred to as round 1 genomic exclusion (4, 5), exconjugants retain their old macronuclei but have identical, homozygous micronuclei. Since macronuclei determine phenotype, each cell retains its old phenotype (including mating type) and is sexually mature. To determine whether wild-type cytoplasm would rescue the cell cycle arrest phenotype, mutant cells were mated with A*III cells or with other mutant cells. Pairs from all matings were isolated into food, and after the pairs came apart, single exconjugants were isolated into drops of food. As controls, unpaired mutant cells and A*III cells were isolated directly from starvation buffer into drops containing growth medium. The drops of growth medium containing single cells (from controls and matings) were shifted to 38°C before any of the single cells divided and incubated overnight. The next day the number of cells in each drop was counted (Fig. 5A).

As expected, drops containing unpaired A*III cells isolated from starvation buffer into food contained numerous cells. Drops containing mutant cells isolated from starvation buffer into food or exconjugants isolated from the mutant-mutant mating all contained single cells after incubation at 38°C. These results show that starvation and/or pairing with cells of a different mating type do not rescue the cell cycle arrest phenotype. Approximately half of the drops containing exconjugants from the group 5 × A*III mating also contained multiple cells. The genotypes of these clones were not tested but they were undoubtedly the A*III exconjugants, since A*III exconjugants retain their original wild-type phenotype after round 1 of genomic exclusion. The other half of the drops containing exconjugants isolated from the mutant × A*III mating contained only a small number of cells whose morphology, at the dissecting microscope level, was indistinguishable from that of group 5 mutants arrested at 38°C. Each drop with mutant cells contained an average of 9.3 cells. Thus, in mutant × A*III pairs, enough rescuing activity passed from the A*III cell to the group 5 cell to support on the average slightly more than three cell divisions.

Cytoplasmic exchange during conjugation is complete. Temporary rescue of the cell division block provided a quantitative means to estimate relative amounts of cytoplasmic exchange. Another experiment was performed to determine whether cytoplasmic mixing is complete during conjugation and to estimate how much rescuing activity each starved cell contains. Round 1 exconjugants from a group 5 × A*III mating were isolated, grown, starved, and remated to give round 2 exconjugants. During round 2, gametic nuclei are produced in both members of the pair, and production of gametic nuclei, reciprocal nuclear exchange, fertilization, and nuclear development occur. Thus, in round 2 a new macronucleus is formed and the genotype of the round 1 micronucleus is expressed as the new phenotype (4, 5). The round 1 exconjugants used in this experiment were both homozygous in the micronucleus for the group 5 mutation. However, one was phenotypically wild type (it was derived from the star strain); the other exconjugant was phenotypically mutant (it was derived from the mutant strain). After round 2, pairs separated, and exconjugants were isolated into drops as described above (except that we kept track of which exconjugants came from which pair) and incubated in growth medium overnight at 38°C. Round 2 exconjugants that successfully completed conjugation developed a new macronucleus homozygous for the group 5 mutation. One round 2 exconjugant made the new macronucleus in a mutant cytoplasm, and the other round 2 exconjugant made

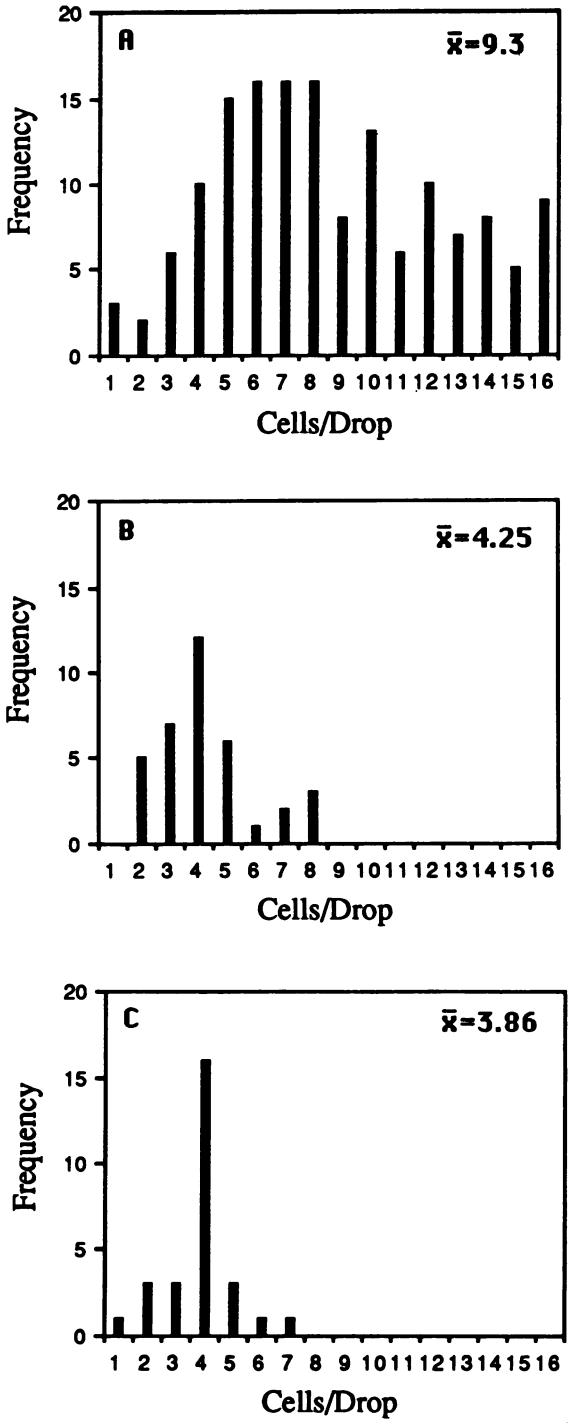


FIG. 5. Cytoplasmic exchange during conjugation transiently complements cell cycle arrest. SG79 and A* cells were mated. Pairs were isolated and allowed to complete round 1 of genomic exclusion at 22°C. Exconjugants were isolated into drops of growth medium and incubated overnight at 38°C, after which the number of cells in each drop was determined (A). Round 1 exconjugants were grown, starved, mated, and allowed to complete round 2 of genomic exclusion and treated as described above (B and C). The histogram in panel B shows the number of mutant cells per drop in cells derived from pairs that had successfully completed round 2 (both exconjugants were mutant). The histogram in panel C shows the number of mutant cells per drop in the mutant cell derived from pairs when round 2 was not completed (the other exconjugant was wild type and underwent numerous divisions).

the new macronucleus in a wild-type cytoplasm. Thus, determining the total number of divisions by both round 2 exconjugants before arrest at 38°C should estimate how much rescuing activity the original wild-type cell contained, while comparing the number of divisions in each member of the pair should estimate the relative amount of cytoplasmic transfer.

In some pairs the cells separated before completing conjugation. One exconjugant from those pairs exhibited wild-type growth at 38°C, while the other exconjugant underwent approximately two divisions before arresting (Fig. 5C). Exconjugants from pairs that completed round 2 of genomic exclusion also divided an average of approximately two times at 38°C (Fig. 5B). If the two members of pairs contained different amounts of the group 5 gene product, we might expect to recover two types of exconjugants: those derived from the round 1 conjugant that had the A*III macronucleus, and those derived from the conjugant with the mutant macronucleus. The histogram shown in Fig. 5B indicates that the exconjugants from the successful round 2 pairings fell into a single class in terms of number of divisions before arrest. Thus, cytoplasmic exchange of the rescuing factor appears to be complete during conjugation. It is interesting that round 2 exconjugants (whether successfully completing conjugation or not) divided fewer times before arrest than did round 1 exconjugants. Round 2 pairs usually stay paired longer than round 1 pairs. If the rescuing factor is not synthesized during conjugation, the decrease in the amount of rescue in round 2 matings could reflect the half-life of the rescuing factor.

DISCUSSION

We have isolated a temperature-sensitive mutation in *T. thermophila* that causes a delay in cilia regeneration, changes in cell shape, arrest at a specific point in the cell cycle, and abnormal nuclear development at 38°C. This mutation probably is not in a gene coding for a component of general metabolism, because cells incubated at 38°C remain motile, maintain approximately the same levels of protein synthesis and of RNAs coding for α-tubulin and actin as wild-type cells at 38°C (Pennock et al., submitted), arrest at a specific point in the cell cycle rather than randomly, and can undergo nuclear divisions during conjugation. These observations suggest that the mutant gene codes for a product specifically involved in cilia regeneration, cell cycle progression, and nuclear development but not nuclear division or DNA replication.

Observations of conjugating cells suggest that an inability to move nuclei properly is the cause of abnormal nuclear development observed in mutant pairs incubated at 38°C. We suggest that an inability to move nuclei at 38°C also causes group 5 cells to undergo cell cycle arrest. We base this suggestion on the following argument. Micronuclear mitosis does not occur in group 5 cells arrested at 38°C. However, since nuclear divisions occur in group 5 cells conjugating at 38°C, it seems unlikely the mitotic apparatus itself is defective in vegetative cells. More likely, some event required for the initiation of mitosis is affected by the group 5 mutation. Prior to normal micronuclear mitosis, the micronucleus elongates slightly, moves away from the macronucleus, and migrates to the cell cortex, where it aligns along a ciliary row (18). The nucleus then elongates and completes mitosis (18). We suggest that the group 5 mutation causes a defect in the machinery that moves the micronucleus from its position adjacent to the macronucleus out to the cell cortex and that this movement is required for micronuclear mitosis. This hypothesis is especially attractive to us because it would explain the seemingly contradictory observations that group 5 micronuclei fail to divide in vegetative cells incubated at 38°C but divide just fine in conjugating cells incubated at 38°C. Thus, it seems likely that the group 5 mutation affects biosynthesis, assembly, or function of microtubules involved in some forms of nuclear movements and cilia regeneration. Regardless of what the group 5 gene product turns out to be, it clearly is involved in the function of at least one microtubule system in *Tetrahymena* (cilia during regeneration) and not in at least one other (microtubules involved in nuclear divisions during conjugation).

It is surprising that this mutation causes true cell cycle arrest in these cells. To our knowledge this is the first cell division cycle (*cdc*) mutation described in *Tetrahymena*. Identification of this gene product should identify an event that triggers or is required for the occurrence of micronuclear mitosis, macronuclear division, cytokinesis, and DNA replication.

Since the group 5 mutation only delays cilia regeneration at 38°C, it might be expected that arrested cells should eventually develop a posterior oral apparatus, but we did not observe this. Many treatments cause both inhibition of cell division and resorption of the oral primordium in *Tetrahymena* if delivered before the transition point (34). It seems likely that any oral primordium that does develop in an arrested cell simply resorbs rather than develops into a visible oral apparatus.

Both abnormal nuclear development and cell cycle arrest can be rescued by wild-type cytoplasm, and a starved, mating cell contains enough rescuing activity to support somewhere between four and six cell divisions. At the end of conjugation both exconjugants contain the same amount of rescuing factor, indicating that cytoplasmic exchange is complete. With recently developed methods for microinjecting *Tetrahymena* (33) and our observations that starved group 5 mutants do not divide when refed at 38°C unless they have received wild-type cytoplasm, it should now be possible to purify the product of this interesting gene.

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